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THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

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INSECTICIDAL CONTROL OF *AGROTIS INFUSA* (Boisd.) AND *LEUCANIA UNIPUNCTA* (Haw.) IN FIELD CROPS.

By A. W. S. MAY, M.Agr.Sc. (Senior Entomologist) and T. PASSLOW, B.Sc.Agr. (Assistant Entomologist), Science Branch, Division of Plant Industry.

SUMMARY.

Several species of cutworms and armyworms are recorded in Queensland, and some of these periodically infest crops in plague numbers. Such infestations by Agrotis infusa **(Boisd.) and** Leucania unipuncta **(Haw.) were experienced during the spring of 1952 on the Darling Downs and in neighbouring districts. Field trials were carried out to determine the optimum DDT dosage for the commercial control of both pests, and several insecticides were screened against** A. infusa.

One-half pound p,p'—DDT per acre effectively reduced larval populations, and ensured yields as high as those following heavier dosage rates. With a lower rate, it is doubtful if the saving in cost would compensate for control breakdown risks involved.

DDT, dieldrin and aldrin were the most efficacious against A. infusa, **with DDT giving most consistent results.**

I. INTRODUCTION.

Several species of cutworms and armyworms attack cultivated plants and pastures in Queensland. The importance of each varies greatly from year to year, prevalence depending to a large extent on locality and seasonal conditions. In some seasons one or more species may occur in plague numbers and severe crop damage follows.

Three cutworms are of major importance. *Euxoa radians* (Gn.) is by far the most prevalent; the density of its spring larval populations is governed by rainfall in the preceding months (Currie 1931). Records of the Department of Agriculture and Stock reveal outbreaks of this pest in all coastal and subcoastal agricultural areas at frequent intervals since the first record in 1907. *Agrotis ypsilon* (Hfn.) is an important pest of vegetables in south-eastern Queensland, and though often found in association with *E. radians* is more tolerant of high soil moisture (Currie 1931).

The third species, *Agrotis infusa* (Boisd.), has been recorded only at infrequent intervals from southern parts of the State. Known as the bogong moth, it is more often recorded as moth plagues in south-eastern Australia (McKeown 1944), and Common (1952) has suggested that larval development

occurs in more northerly regions. Larval plagues have been recorded in southern Queensland on several occasions since 1901. During a widespread outbreak in 1931, larval plagues were experienced in August as far west as Cunnamulla, while later in the same year adults swarmed in tomato fields and infested dwellings in the Brisbane district (unpublished Departmental records). The most recent outbreak occurred in July and August of 1952, when larvae damaged a wide range of crops on the Darling Downs (for example, see Fig. 1), and in the south-western agricultural districts.

Fig. 1.

A Poor Stand of Wheat Caused by the Cutworm *Agrotis infusa* (Boisd.).

Other cutworms, including *Agrotis poliotis* (Hmps.), *A. tibiata* Gn., *A. porphyricollis* Gn. and *Mocis frugalis* (F.), damage crops from time to time but these are seldom of economic importance.

The most important armyworm, *Leucania unipuncta* (Haw.), occurs in most coastal and subcoastal districts, where it may damage pastures, sugar-cane and cereal crops. Outbreaks of this pest have been recorded at frequent intervals since 1899 in various parts of the State (unpublished Departmental records). On the Darling Downs and in neighbouring districts minor attacks may be experienced in spring and autumn (Passlow 1952), but when large pest populations are present, chiefly in the spring months, widespread crop damage results. Plagues occurred in these districts in 1931, 1938, 1948 and 1952.

Relatively infrequent plagues of three other species—*Leucania loreyi* (Dup.), *Laphygma exempta* (Wlk.) and *Spodoptera mauritia* (Boisd.)—have occurred in coastal districts (Jarvis 1927; Smith 1933; Weddell 1936). *Leucania diatrecta* Butl. in small numbers is sometimes associated with *L. unipuncta*.

In the past, control of cutworms and armyworms was usually undertaken with Paris green or arsenic pentoxide baits and mechanical barriers (Currie 1931; Weddell 1936; Sloan 1937). The labour entailed in mixing and distributing the baits, the erratic availability of materials and the difficulties of obtaining control over large areas have suggested the need for other methods. The introduction of boom spraying and newer insecticides presents an alternative approach to the problem of control in field crops. A provisional recommendation, following reports in overseas literature (e.g., Luginbill 1950), of 1 lb. of *p,p'*—DDT per acre has given staisfactory kills in Queensland. Increasing costs, however, indicated the necessity for dosage level and yield trials, and the 1952 plagues of *A. infusa* and *L. unipuncta* in various crops on the Darling Downs afforded an opportunity for this work. At the same time, several newer insecticides were screened against *A. infusa*.

As the habits and food preferences of the several species of cutworms and armyworms mentioned are somewhat similar, the findings in these trials might reasonably be expected to have general application.

II. AGROTIS INFUSA (BOISD.).

Three DDT dosage level trials, two in lucerne and one in wheat, and two screening trials in lucerne and barley, were carried out against this cutworm.

Materials.

The following insecticides were used:—

Aldrin—An emulsifiable preparation containing 20%, w/v, active ingredient.

BHC—A dispersible powder containing 6·5% gamma isomer.

Chlordane—An emulsifiable preparation containing 80%, w/v, active ingredient.

DDT—an emulsion concentrate containing 25% *p,p'* isomer w/v.

Dieldrin—An emulsifiable preparation containing 16%, w/v, active ingredient.

Parathion (E605)—An emulsifiable preparation containing 25%, w/v, active ingredient.

All insecticides were applied by knapsack at the rate of 100 gallons per acre, but each application rate is expressed as weight of active ingredient per acre.

The various treatments are detailed in the tables of results.

Designs of Trials.

Dosage level trials were set out as 4 x 6 randomized blocks, while screening trials conformed to an 8 x 3 layout. In all trials, a plot size of 1/100th acre was used.

Assessing Results.

Larval Mortality and Survival.—Following spraying, estimates of mortality were usually made by counting the number of dead larvae observed in two or three spaces across each plot, a space being the soil surface between two adjacent crop rows. The period between spraying and the first count was two to three days. A further count was taken in two of the trials two days later to check residual activity of the insecticides.

In one screening trial in barley (Cambooya), efficacies of treatments were determined by the mortalities among 60 live larvae collected at random from each plot two days after treatment. Larval samples supplied with food from the respective plots were held for three days in strong paper bags, when counts of live larvae were made.

In the dosage level trial on lucerne (Glenvale), as well as estimating mortality, a count was made one week after treatment of larvae and pupae surviving in the upper four inches of soil in six foot-square quadrats per plot selected at random.

The data for larval mortality and survival per plot are presented as both the equivalent and transformed means, the latter being calculated by using either the $\sqrt{x+\frac{1}{2}}$ or the $\sqrt{x}$ transformation.

Yields.—Yields were taken in four of the five trials and are expressed in economic units. The lucerne trials were cut with a hand-operated power mower, and the green weights recorded. Following grain ripening, wheat plots were mown, the bulk weighed and then threshed for grain weights in a small stationary threshing machine.

RESULTS.

Level Trials.

Lucerne—Regional Experiment Station, Hermitage.

Considerable foliage damage had already occurred in this seedling crop when treatments were applied on August 12, though no actual thinning of the stand was noticeable. Good growing conditions had prevailed prior to infestation by the cutworms and substantial crown development had taken place. Feeding was concentrated on the leaves and softer stems. It was noticeable that feeding virtually ceased in all treated plots.

Counts of dead larvae were made three days after treatments. For plot yields, the first cut was made on September 23 and 24 shortly before flowering commenced, and the second approximately six weeks later at peak flowering.

The results are given in Table 1.

Table 1.

MORTALITY DATA AND PLOT YIELDS.

(Lucerne—Hermitage.)

Treatment.	Dead Larvae per Plot.		Mean Yield per Plot (tons/acre).		
	Equivalent Mean.	Transformed Mean ($\sqrt{x}$).	First Cutting.	Second Cutting.	First plus Second Cutting.
DDT 1lb./ac.	44·1	6·64	2·05	3·15	5·21
DDT ¾lb./ac.	40·8	6·39	1·98	3·25	5·22
DDT ½lb./ac.	37·1	6·09	2·09	3·14	5·24
No treatment	2·56	*	1·11	3·04	4·15
Differences necessary for 5% significance	..	·77	·24	·31	·38
Differences necessary for 1% significance	..	1·10	·33	·34	·53

* As values for all DDT treatments obviously exceeded that for no-treatment, the atter was omitted from this analysis.

Though no differences were found between the three levels of DDT application, spraying produced more than an 80% increase in yield for the first cutting. This benefit did not continue, as the crop recovered before the second cutting.

Lucerne—Glenvale District.

Though a seedling crop, the lucerne was more advanced than the stand used for the previous trial. When treatments were applied on August 22, considerable leaf and stem damage had been caused by a large population of late instar larvae.

Counts of dead larvae in three inter-row spaces per plot were made three and five days after treatment. Larval survival was estimated one week after treatments were applied. These data are presented in Table 2.

The trial was harvested on November 3 and 4, and the mean weights of green lucerne are also in Table 2.

For larval mortality, all treatments significantly exceeded no treatment. Greater numbers of both larvae and pupae were alive in no-treatment plots than in any of those receiving DDT, but no significant differences were found for this criterion between DDT treatments. Migration of larvae from the surrounding untreated lucerne into the trial area had occurred, and this would account for the apparent survival in treated areas. This movement of larvae would also tend to reduce yield differences due to treatments.

Table 2.

MORTALITY AND YIELD DATA.

(Lucerne—Glenvale.)

Treatment.	Dead Larvae per Plot.				Survival per Plot.		Yield (tons/ acre).
	3 days.		5 days.				
	Transformed Mean.*	Equivalent Mean.	Transformed Mean.*	Equivalent Mean.	Transformed Mean.*	Equivalent Mean.	
DDT 1lb./ac.	5·63	31·2	4·87	23·2	2·50	6·25	3·02
DDT ½lb./ac.	5·32	27·8	4·42	19·0	2·72	7·40	3·00
DDT ¼lb./ac.	3·74	13·5	3·48	11·6	2·67	7·13	2·78
No treatment	1·53	1·8	1·36	1·3	4·22	17·81	2·88
Differences necessary for significance 5%	1·15	..	·99	..	·94	..	·47
Differences necessary for significance 1%	1·58	..	1·37	..	1·30	..	·65

* The transformed means in columns 2 and 4 were calculated from the $\sqrt{x+\frac{1}{2}}$ transformation, whilst for larval survival (column 6) the $\sqrt{x}$ transformation was used.

Differences in growth rates between treated and untreated plots were noticeable shortly after treatments were applied, but were not maintained. Prolonged wet weather late in the growing period promoted rapid growth, so at harvest significant yield differences were not obtained.

Wheat—Bowenville District.

A large population of late instar larvae was present when treatments were applied on August 14. At this stage, the crop, approximately four inches in height, had been thinned considerably, while further damage was expected in no-treatment plots.

Because of continuous dry, hot weather during the flowering and grain setting period, yields were low at harvest on November 24, while the grain was pinched severely. Only yield data, bulk (grain and straw) and grain weights, were obtained for this trial. Table 3 gives the mean values in each category.

Table 3.

YIELD DATA.

(Wheat—Bowenville.)

Treatment.	Bulk Weight. (lb. per plot).	Grain Weight. (bus./acre).
DDT 1lb./ac.	26·2	11·4
DDT ¾lb./ac.	25·5	10·9
DDT ½lb./ac.	26·0	11·6
No treatment	21·6	9·0
Differences necessary for significance 5%	1·9	1·4
Differences necessary for significance 1%	2·6	2·0

Despite the weather conditions, all DDT-treated plots yielded significantly better than no-treatment plots for both bulk and grain weights. No differences were found, however, between the three levels of DDT application.

Screening Trials.

Lucerne—Glenvale District.

Treatments were applied in this trial, adjacent to the DDT level trial on lucerne (Glenvale), on August 23. Counts of dead larvae in three spaces per plot were made two and four days later. The plots were harvested on November 4 and 5.

The data for larval mortality and yields are given in Table 4.

The first three treatments listed in Table 4 were significantly better than the remainder for the first count, although the differences were not so marked two days later. Larval migration was evident from the surrounding unsprayed crop as well as between plots and would account in part for this levelling. DDT was significantly better than dieldrin on the earlier counting only. This finding is in accord with results from trials with these two insecticides against other noctuid larvae (unpublished Departmental records).

Table 4.

LARVAL MORTALITY AND PLOT YIELDS.

(Lucerne—Glenvale.)

Treatment.	Dead Larvae per Plot.				Yields (tons/acre).
	2 days.		4 days.		
	Transformed Mean.*	Equivalent Mean.	Transformed Mean.*	Equivalent Mean.	
DDT ½lb./ac.	4·94	23·9	3·11	9·17	3·17
Dieldrin ½lb./ac.	3·52	11·9	3·45	11·40	2·81
Aldrin ½lb./ac.	4·34	18·3	2·87	7·74	2·81
Chlordane ½lb./ac.	1·64	2·2	1·72	2·46	2·86
Toxaphene ½lb./ac.	1·68	2·3	1·27	1·11	2·70
BHC ⅕lb./ac.	1·79	2·7	1·56	1·93	2·74
Parathion $\frac{1}{10}$lb./ac.	1·78	2·7	1·68	2·32	2·68
No treatment	1·10	·7	1·29	1·16	2·95
Differences necessary for significance 5%	1·13	..	1·20	..	·54
Differences necessary for significance 1%	1·57	..	1·67	..	·75

* $\sqrt{x+\frac{1}{2}}$

Numbers were not unduly high in this trial, but the differences in larval mortalities between the first three treatments and the others were reflected in crop growth soon after spraying. Good rains and excellent growing conditions during the later stages of crop growth helped to nullify these earlier differences.

Barley—Cambooya District.

When treatments were applied on August 13, the barley was approximately 10 inches high and a large population of late instar larvae was present.

Yield data were not obtained in this trial. The stand had been so depleted by the cutworms before treatments were applied that worthwhile differences between plots either for green weight of crop or grain yield could not be expected.

The data for larval survival per plot are set out in Table 5.

Table 5

MEAN NUMBER OF LARVAE SURVIVING PER PLOT.

(Barley—Cambooya.)

Treatment.	Transformed Mean.*	Equivalent Mean.
DDT ½lb./ac.	1·38	1·90
Dieldrin ½lb./ac.	1·91	3·65
Aldrin ½lb./ac.	2·66	7·08
Chlordane ½lb./ac.	4·62	21·34
Toxaphene ½lb./ac.	5·00	25·00
BHC ¼lb./ac.	5·03	24·30
Parathion $\frac{1}{10}$lb./ac.	4·86	23·62
No treatment	5·19	26·94
Differences necessary for significance { 5%	·63	..
Differences necessary for significance { 1%	·88	..

*$\sqrt{x}$

It was evident when counts were made three days after field sampling that escape losses and attacks of imprisoned larvae on one another had occurred in all samples except those from the first three treatments listed in Table 5. The figures for this small group are a fair indication of efficacies, whereas for the others the values as indicated by the counts are low. Less than half the number of larvae collected from no-treatment plots were present in the containers at the final counting.

DISCUSSION.

Level Trials.

Marked yield differences between treated and untreated plots were obtained following DDT applications during early plant growth, but yield improvements were not so marked when treatments were applied late. This was borne out in the lucerne trials at Hermitage and Glenvale, though other factors also influenced results. The cutworm population was much higher in the Hermitage trial, and commercial spraying of the surrounding areas prevented larvae migrating onto the trial site.

There were no significant differences in either kills or yields following dosages of DDT from ½ lb. to 1 lb. per acre. When ¼ lb. per acre was used quite a satisfactory kill was obtained, but it was lower and slower than those by the higher dosages. Furthermore, there was some evidence that following sub-lethal doses of DDT, larvae are repelled by the insecticide. In most instances an application of ¼ lb. DDT per acre would be followed by further crop damage. On a cost basis, however, it is doubtful if the relatively small saving in material would compensate for the possible breakdown in control which might occur.

Screening Trials.

At the dosage rates used in these trials, DDT, dieldrin and aldrin will control cutworm infestations, but DDT gave most consistent results. Some of the formulations did not wet the plants properly, but deposits on the soil surface generally and at plant bases should have ensured contact with the larvae.

III. LEUCANIA UNIPUNCTA (HAW.).

Three trials were established against late instar larvae to determine the most effective dosage of *p,p'*–DDT, and the levels tested are given in the tables of results. Method of application and quantity of spray applied per plot were similar to those for the cutworm trials.

Designs of Trials.

The autumn trial (on oats at Wyreema) comprised a 5 x 4 randomized layout with plots in line along the face of a heavy infestation of migrating larvae.

Both spring trials, also on oats, consisted of 4 x 6 randomized blocks. The first, at Wellcamp, was set out in a uniformly infested crop, though in the second, at Biddeston, plots were arranged in line, ahead of migrating larvae.

In all trials, a plot size of 1/100th acre was used.

Assessing Results.

Data were obtained in terms of plant stand counts, laboratory and field estimates of larval kills, and plot yields.

Stand Counts.—In the autumn trial (Wyreema), the number of plants in five-row sections, each 10 feet long, was recorded just prior to and again one week after treatment. All plants visible above ground were counted.

Larval Mortality and Survival.—From 50 to 100 larvae were collected at random from each plot 24 hours after treatment. Samples were held in strong paper bags for several days, when the numbers alive and dead at various intervals after treatment were recorded. All larvae showing movement on each counting day were classed as alive. In the laboratory, the larvae from

the autumn trial were fed on insecticide-free plant material, while those from the two spring trials were fed on plants from the respective plots from which the samples were taken.

Field estimates of mortality, similar in method to that employed in the cutworm trials, were made in one spring trial (Wellcamp). The number of dead larvae observed in three spaces across each plot was recorded after treatment.

The data for larval mortality and survival per plot are presented as both the equivalent and the transformed means. When presented as numbers of dead larvae per plot, the latter was calculated using the transformation $\sqrt{x + \frac{1}{2}}$. When expressed as percentage mortality or survival, the inverse sine transformation was used to equalise the variances.

Plot Yields.—The Wyreema trial, conducted during a small outbreak in the autumn, was not harvested. Damage to the untreated experiment plots and the remainder of the commercial crop in the same field (Fig. 2) was so extensive that the field was prepared for replanting soon after mortality data and stand counts were recorded.

Fig. 2.

The DDT Dosage Level Trial Against *Leucania unipuncta* (Haw.) at Wyreema. Larvae entered the field from the left of the photograph. The denuded bands within the trial are the untreated plots.

At grain maturity, the spring trials were mown. In the Wellcamp trial, both weight of bulk material and grain per plot were recorded, the grain being threshed out in a small stationary threshing machine. For the Biddeston

trial, only grain weights were recorded, the bulk material being passed through a field header. This proved unsatisfactory for experimental purposes. Yields are expressed in economic units.

RESULTS.

Autumn Trial.

Oats—Wyreema District.

Large numbers of armyworms were migrating into the newly germinated crop when treatments were applied on May 1. The stand counts and percentage larval mortality for treatments are given in Table 6.

Table 6.

PLANT STAND COUNTS AND LARVAL MORTALITY.

(Oats—Wyreema.)

Treatment.	Plant Stand Count.			Larval Mortality (percentage).	
	Pre-treatment.	Post-treatment.		Transformed Mean.	Equivalent Mean.
	Mean.	Mean.	Adjusted Mean.*		
DDT 1lb./ac.	82·0	103·6	104·2	77·4	95·3
DDT ¾lb./ac.	85·2	99·4	98·6	69·9	88·2
DDT ½lb./ac.	73·0	92·9	97·6	63·5	80·1
DDT ¼lb./ac.	84·8	96·1	95·5	59·1	73·6
No treatment†	92·0	66·8	62·9	38·0	37·9
Differences necessary for significance 5%	16·5	..	10·1	10·1	..
Differences necessary for significance 1%	23·1	..	14·2	14·2	..

* The post-treatment mean has been adjusted following examination of the error regression of post-treatment on pre-treatment counts.

† Data for no-treatment plots, which were obviously inferior (see also Fig. 2), were not included in the analysis.

All DDT treatments effectively prevented further damage. The recorded mortality in no-treatment plots was due to larval immigration after treatments were applied.

Spring Trials.

Oats—Wellcamp District.

Armyworms were distributed throughout this crop when treatments were applied on September 3. At this time, plants were 12 inches high and had tillered considerably following earlier grazing. Extensive flag injury was apparent.

Counts of dead larvae were made one day after treatments, while percentage larval survival among collected samples was estimated five and seven days after sampling. The trial was harvested on November 21, when bulk and grain weights were recorded.

The data are set out in Table 7.

Table 7.

LARVAL MORTALITY AND YIELD DATA.

(Oats—Wellcamp.)

Treatment.	Larval Mortality (number).		Larval Survival (percentage).				Yield.	
			5 days.		7 days.		Bulk Weight (lb./plot).	Grain Weight (lb./plot).
	Transformed Mean.	Equivalent Mean.	Transformed Mean.	Equivalent Mean.	Transformed Mean.	Equivalent Mean.		
DDT 1lb./ac. ..	7·97	63·0	21·1	13·1	7·8	1·8	37·8	30·8
DDT ½lb./ac. ..	6·10	36·7	31·8	27·8	19·1	10·7	32·8	27·6
DDT ¼lb./ac. ..	6·16	37·4	46·0	51·7	36·2	34·9	36·1	30·3
No treatment ..	1·45	1·6	83·4	98·2	74·4	92·8	30·9	24·0
Differences necessary for significance 5%	·80	..	5·2	..	7·7	..	4·7	2·7
Differences necessary for significance 1%	1·10	..	7·2	..	10·6	..	6·5	3·7

The highest dosage rate was superior to the others and destroyed a high percentage of larvae soon after spraying. Indifferent results were obtained with the two lower dosage rates within 24 hours of application, but the efficacies of these treatments increased considerably in the later count for larval survival. The gross plant cover prevented effective spray penetration and it could be expected that at the lower dosage rates a longer period would elapse before larvae contacted a lethal dose of insecticide.

Prior to head emergence, differences in crop growth were obvious between treatments. Unusually high rainfall during the grain setting period promoted growth and reduced plot differences. Despite this, worthwhile yield increases due to spraying were obtained in weight of grain.

Oats—Biddeston District.

Treatments were applied on September 10, when the oats was approximately 18 inches high. At this time, late instar larvae were entering the crop from an adjacent field of barley.

Larval survival was determined one and four days after treatments were applied.

Harvesting was carried out on November 19, and relevant data are presented in Table 8.

No significant difference was found between values for the higher levels of DDT four days after spraying, but both gave significantly better results than the lower rate of application.

Table 8.

LARVAL SURVIVAL AND YIELD DATA.

(Oats—Biddeston.)

Treatment.	Larval Survival (percentage).				Yield (bus./acre).
	1 day.		4 days.		
	Transformed Mean.	Equivalent Mean.	Transformed Mean.	Equivalent Mean.	
DDT 1lb./ac.	40·8	42·6	21·9	13·9	37·0
DDT ½lb./ac.	46·7	52·9	27·0	20·6	37·6
DDT ¼lb./ac.	54·8	66·7	39·0	39·6	30·7
No treatment	85·2	99·3	77·7	95·5	32·2
Differences necessary for 5% significance	8·8	..	7·8	..	11·8
Differences necessary for 1% significance	12·2	..	10·8	..	16·5

Differences between larval survival for treatments at four days are not reflected in the yield data. The method of grain recovery (see pp. 10-11) and excessive rain during grain setting probably reduced differences that were apparent between treatments at head emergence.

DISCUSSION.

Though larval feeding was prevented almost entirely by spraying, high mortality was obtained only with the higher dosages of DDT. Kills were slow when DDT was used at ½ lb. per acre, but four days after application these were comparatively satisfactory.

Despite a slower killing rate and differences in larval mortality, significant differences in yields between dosage were not obtained. This suggests that all rates of DDT application were effective in reducing the activities of pest populations to below the level at which economic crop damage occurs. When ¼ lb. DDT per acre was used a proportion of final instar larvae was not prevented from pupating normally.

IV. GENERAL COMMENTS AND RECOMMENDATIONS.

General field observations also indicate that the extent and rapidity of damage by cutworms and armyworms depend to some extent on the age of the crop attacked. This was particularly evident with infestations of *A. infusa* in lucerne prior to crown development, as the plants were destroyed, necessitating replanting. More advanced crops though defoliated by these pests usually recovered sufficiently to give worthwhile yields at the second cutting. Similarly in cereals, depletion of stands occurred only when crops were infested in the seedling stage. Damage by cutworms and armyworms after the development of the main root system and subsequent tillering was usually confined to defoliation. Armyworms, however, sometimes caused serious

reduction in yields when late instar larvae chewed through the stems just below the heads. This occurred after the plants had been defoliated, or the leaves were drying out with plant maturity.

Based primarily on the data from these trials, the use of DDT, preferably in emulsion form, applied at the rate of one-half pound of active ingredient per acre, was recommended for the controls of cutworms (May 1953) and armyworms (Passlow 1952) in field crops. The importance of spraying at the first signs of damage was stressed.

Results from the wider commercial use of these recommendations during 1952 and 1953 amply confirmed the findings presented in this paper.

V. ACKNOWLEDGMENTS.

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MANGANESE TOXICITY IN AN ACID SOIL.

By I. F. FERGUS, B.Sc., Analyst, Plant Nutrition Section, Chemical Laboratory, Division of Plant Industry.

SUMMARY.

The occurrence of manganese toxicity in French bean gown on an acid soil (referred to as the Dagun soil) is reported.

The manganese content of the plants in the field vdries with the pH of the soil, reaching 3,000 ppm at pH 4.4 Healthy plants contained between 200 ppm and 1,000 ppm manganese, and values greater than 1,000 ppm may be regarded as indicating manganese toxicity.

The amount of exchangeable manganese in the soil is not a reliable criterion for predicting manganese toxicity, but if the pH is low and the easily reducible manganese high, the soil can be regarded as suspect.

French bean is a good indicator plant for excessive manganese in the soil.

A pot trial indicated that the excessive uptake of manganese by French bean grown on the Dagun soil can be controlled by applying lime to raise the pH to about 5.4.

Some evidence is presented that pineapples grown on the Dagun soil and peanuts grown on certain other soils suffer from manganese toxicity.

INTRODUCTION.

Manganese toxicity in plants grown on strongly acid soils rich in manganese has been reported by various authors. Lohnis (1951) described experiments with French beans (*Phaseolus vulgaris*) and other crops in the Netherlands. Sherman and Fujimoto (1946) found up to 3,800 ppm manganese in affected lettuce plants in Hawaii. Somers and Shive (1942) studied the iron and manganese contents of soybeans and concluded that a relationship existed between the ratio of soluble iron to soluble manganese and the occurrence of injury in the soybeans. Parberry (1943) reported that beans grown in certain soils in New South Wales were affected by excess manganese, and found the effects to be more marked when associated with magnesium deficiency.

OCCURRENCE IN QUEENSLAND.

The main soil type in Queensland which has given rise to manganese toxicity problems occurs in the south-east of the State, near Dagun, which is situated on the edge of the Mary River Valley. It will be referred to in this paper as the Dagun soil. This soil is used mainly for the growing of pineapples and French beans. In work to be described, beans were selected as the indicator crop, but pineapples are also affected by the disorder.

The soil type is developed from a jasper, which occurs in the district interbedded with cherts and shales of Devonian age. Deposits of manganiferous ores occur in various parts associated with the jaspers, and these have been mined for manganese. According to W. C. Burns (unpublished data, 1951), the manganese was originally deposited with the sediments of the enclosing formations in a relatively deep water environment. Compaction and induration resulted in the formation of haematitic coloured shales, together with

manganiferous radiolarian jaspers and cherts. Subsequent major strike faulting caused shattering of these rocks, and facilitated the leaching of the manganese minerals, either to fill open fractures in the shales or to cement and replace shattered cherts and jaspers. This resulted in the formation of localised deposits rich in manganese. The manganese remaining in the rocks and passing to the soil after weathering has been of sufficient concentration to cause manganese toxicity problems.

The area receives predominantly summer rainfall, with a moderately dry winter. Gympie, about 10 miles from Dagun, receives an average annual rainfall of 45·43 inches, distributed as follows:—

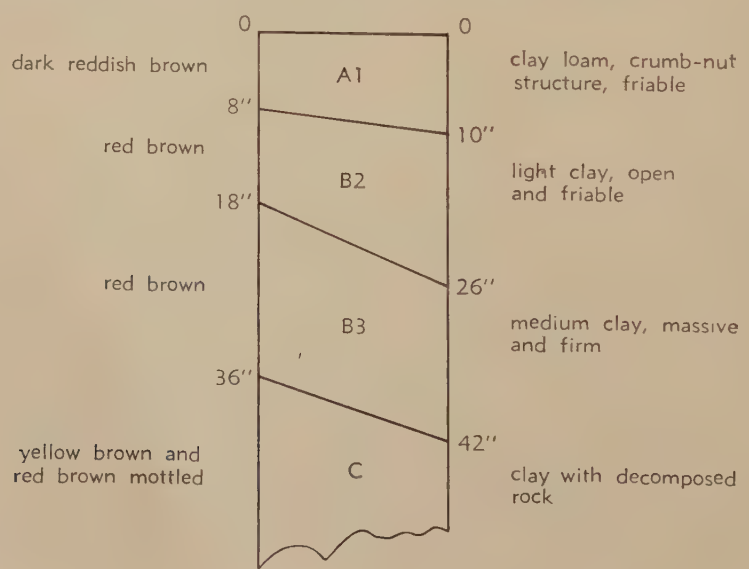
January-March	19·28 in.
April-June	8·94 in.
July-September	5·74 in.
October-December	11·46 in.

The rainfall for Dagun might be assumed to be a little higher but similarly distributed. The low winter rainfall is unreliable, so that irrigation is necessary for a winter crop of beans and is used by most farmers.

In summer, both temperature and humidity are high, while in winter, frosts may be severe. Most of the bean crops, however, are grown at an elevation sufficient to escape serious frost damage.

THE SOIL SERIES.

In the virgin state the soil, which belongs to the great soil group "Kratznosem", is a dark reddish brown clay loam on the surface, fairly high in organic matter, with a good crumb structure. It has been designated the Dagun Series. The profile description of the virgin soil is:—



Steep slopes are used for cultivation. This has been practised for a number of years; consequently in many parts the structure of the surface soil has deteriorated and erosion has reduced the depth of the soil. The organic matter content has also been considerably reduced and aeration is now poor because of these factors.

The greatest change in the properties of the soil as a result of cultural practices has been in its reaction. The pH value (soil to water ratio 1:2·5) of the surface horizon in the virgin soil is approximately 6·0, while that of the cultivated area is now as low as 4·2 in some portions. Part of this increase in acidity is due to cultivation practices and part to the heavy fertilizer applications (mainly sulphate of ammonia) which are used for pineapples.

SYMPTOMS OF MANGANESE TOXICITY.

These are almost identical with those described by Lohnis (1951) and with colour plates illustrated by Wallace (1951). Beans grown on the very acid parts of the area are stunted and show severe marginal and interveinal chlorosis in the leaves. In extreme cases, the leaves are crinkled and exhibit necrotic spotting. Very little flowering then occurs and practically no fruit is set. Less severely affected plants may recover somewhat as they approach mtaurity, but the yield of pods is poor.

PLANT ANALYSIS.

Analysis of unthrifty plants offers some proof that excessive manganese uptake is the cause of the trouble. The plants were digested with perchloric, nitric and sulphuric acids, and the manganese determined colorimetrically after oxidation to permanganate with potassium periodate, using the method of Willard and Greathouse (1917). The colours were read in a Lumetron photoelectric colorimeter using an M515 filter and 1 cm. cells.

Affected plants were found to contain up to 3,000 ppm manganese on a dry-matter basis. Healthy plants, on the other hand, varied between 200 and 1,000 ppm. Some values for manganese in the whole plant, and the soil pH at which the plants were grown, are given in Table 1.

Table 1.

PLANT GROWTH IN RELATION TO SOIL pH AND MANGANESE CONTENT OF PLANT.

Soil pH.	Mn in Plant (ppm).	Plant Growth.
4·4	3000	Very poor
4·5	2400	Poor
4·8	1200	Fair. Some chlorosis
4·9	800	Good
5·0	1100	Fair. Some chlorosis
5·9	260	Good

Fig. 1 illustrates the relationship between manganese in the plant and soil pH, and demonstrates the influence of pH on manganese uptake by beans.

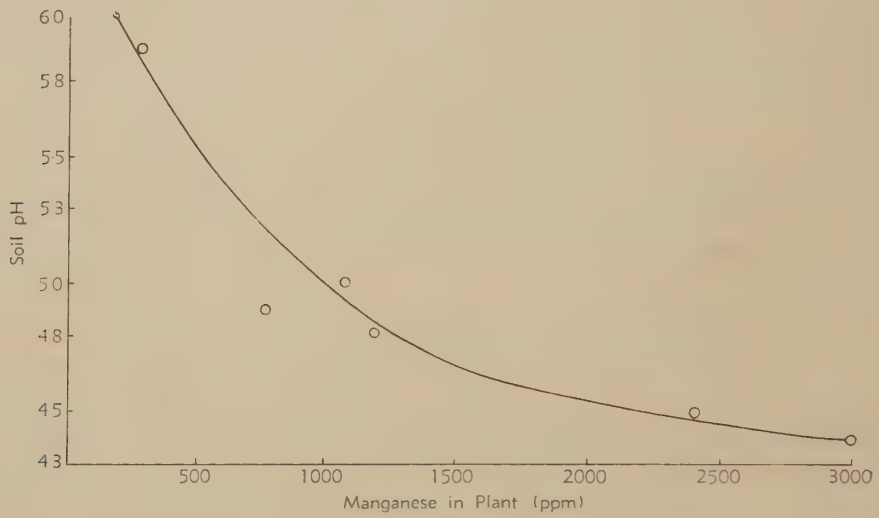


Fig. 1.

Correlation Between Manganese in Plant and Soil pH.

SOIL ANALYSIS.

Manganese in the soil can exist in varying degrees of oxidation, the forms being in dynamic equilibrium with one another. The oxidation-reduction status is affected by several factors, of which pH and organic matter are the most important. The bivalent manganous ion is oxidised by soil organisms to manganic forms of higher valency when the pH is greater than 5·5; the reverse can also occur, the higher oxides being reduced either by biological processes or by reaction with organic matter. Leeper (1947) pointed out that the reduction with organic matter is the more likely at lower pH values, because of the rapid increase in the oxidizing powers of the higher oxides as the pH falls.

The manganous form is usually regarded as being available to the plant, and Piper (1931) maintained that the amount of manganous manganese present in the soil is an indication of likely deficiency in the plant. Leeper (1935, 1947) laid emphasis on the higher oxides, and considered it important to examine the oxidation-reduction state of the manganese. He divided the manganic oxides (the formula for these being empirically written as MnO_2) into highly reactive forms, which could be easily reduced by quinol, and the less active forms which could react with sodium thiosulphate. The remainder of the higher oxides of manganese in the soil is regarded as inert. That portion of the active manganic oxides which can be reduced with quinol he referred to as the "easily reducible manganese".

The manganous form in the soil is usually termed exchangeable manganese, and is determined by extraction with a neutral salt. Ammonium acetate is commonly used, but Heintze (1938), used calcium nitrate, and this method was followed by Lohnis (1951). Leeper (1935), investigating manganese deficiency in soils, preferred to determine the "easily reducible manganese", because some soils could not maintain a supply of the exchangeable form but plants were still healthy when grown on them. He used a 0·2% solution of quinol in normal ammonium acetate as the extractant. Later Jones and Leeper (1951) used a less drastic method of extracting the easily reducible manganese, and obtained a better correlation with the occurrence of manganese deficiency in plants. They treated the soil with 0·05% quinol in 50% alcohol, centrifuged, and then extracted the reduced manganic oxides from the soil with 0·5 M calcium nitrate. Sherman (1942), adapted Leeper's original method and determined water soluble, exchangeable and easily reducible manganese to give an indication of the manganese status of the soil.

Most of these methods have been applied to neutral or alkaline soils as a means of testing for manganese "deficiency", but their usefulness with acid soils as a means of testing for manganese "toxicity" is doubtful.

The problem was approached first with a view to correlating soil pH with plant growth, and next with a view to predicting the uptake of manganese by plants by determination of the exchangeable and the easily reducible manganese. Fig. 1 demonstrates a relationship between plant manganese and soil pH, and Table 2 compares the pH of surface soils taken from various parts of the affected area with the observed growth of beans.

Table 2.

pH of Surface Soil and Observed Growth of Beans.

Soil pH.	Plant Growth.
4·4	Very chlorotic
4·7	Very chlorotic
4·7	Chlorotic
4·9	Very chlorotic
4·9	Fair. Some chlorosis
5·1	Good
5·7	Good. A few plants affected
6·1	Good

Dolomite had been applied on the area from which the pH 5·7 sample was taken, and uneven distribution of the dolomite probably accounts for the few affected plants. These figures indicate that at a pH of greater than 5·0 good growth can be expected. If the pH is less, the plants will probably show symptoms of toxicity.

Exchangeable manganese was determined by extraction with neutral N ammonium acetate for 30 minutes, following the method of Sherman, McHargue and Hodgkiss (1942). Good correlation between pH and exchangeable manganese was obtained. The results are given in Table 3 and represented graphically in Fig. 2.

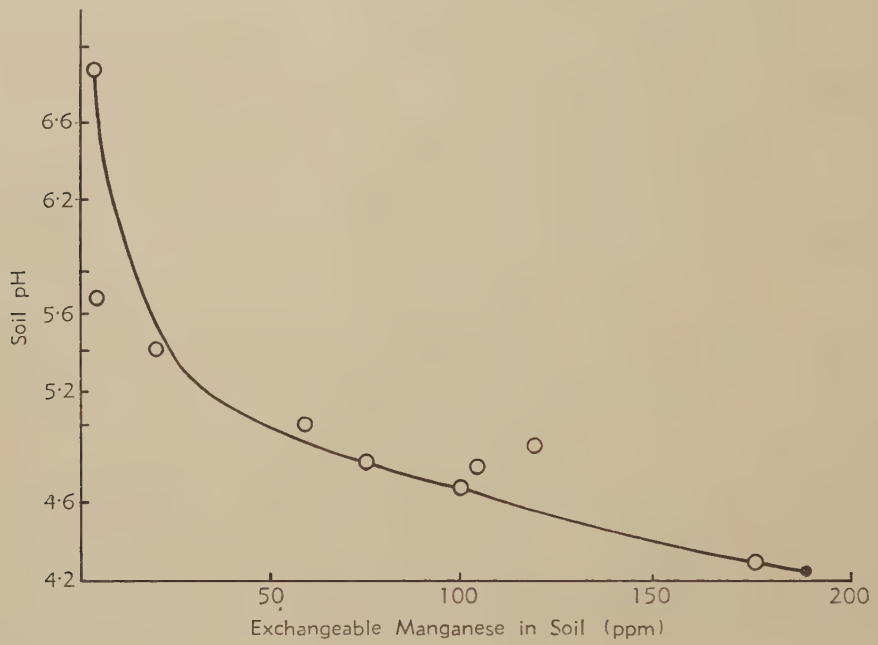


Fig. 2.

Correlation Between Exchangeable Manganese in Soil and pH.

The sharp drop in the amount of exchangeable manganese when the soil pH varies from 4·9 to 5·4 is consistent with the figures given in Table 2.

The easily reducible manganese was determined by extracting the soil with 0·2% quinol in N ammonium acetate solution. Large amounts were extracted from all samples, even when the pH was quite high. The method of Jones and Leeper (1951) was also used for a few samples, but high values were still obtained at different pH levels. The results are given in Table 3.

The inference is that the Dagun soil has a large reserve of active manganese, which can be reduced to the exchangeable form when conditions are favourable. From the ratio of exchangeable to easily reducible manganese it appears that only a relatively small fraction is reduced at any one time. For this soil type the amount of exchangeable manganese indicates to some extent whether or not the plant will take up an excess of this element.

Table 3.

EXCHANGEABLE AND EASILY REDUCIBLE MANGANESE IN DAGUN SOIL.

Soil pH.	Exchangeable Manganese.	Easily Reducible Manganese (0·2% Quinol).	Easily Reducible Manganese (Jones & Leeper).
	ppm	ppm	ppm
4·3	175	2080	
4·4	105	2600	1720
4·5	104	3400	
4·5	74	2550	1620
4·7	100	2180	1470
4·9	120		
5·0	62		
5·4	20	2700	
5·7	2	2750	1680
6·9	2		

Leeper (1947) has shown the easily reducible fraction of the active manganese to be important for assessing the status of soils which are deficient in manganese, but its importance for the Dagun soil with a toxicity problem is doubtful. Areas of the Dagun soil with reaction values of 5·5 or higher contain large amounts of reducible manganese, which does not appear to affect the beans grown on them. It would seem that this fraction of the manganese is not available to the plant.

It is interesting to record here some further analytical data relating to the active manganese content of soils from four other districts. Values for these are given in Table 4.

Table 4.

ACTIVE MANGANESE CONTENT OF VARIOUS FARM SOILS.

District.	Soil pH.	Exchangeable Manganese.	Easily Reducible Manganese.	Comments.
		ppm	ppm	
Brookfield ..	6·3	2	940	
Maryborough 1...	5·7	6	14	
Maryborough 2...	7·5	2	38	
Coolabunia 1. ..	4·3	200	520	Poor peanut growth
Coolabunia 2. ..	4·8	10	1360	Fair peanut growth
Coolabunia 3. ..	5·3	46	450	Good peanut growth
Beaudesert ..	6·0	0·5	80	Responded to applications of Mn

The poor growth of peanut (*Arachis hypogea*) on a soil from Coolabunia is probably due to manganese toxicity. The soil from Brookfield could give rise to toxicity problems should the reaction be allowed to become very acid.

Pot Trial.

To demonstrate the effect of lime on plant growth and on exchangeable manganese of the soil, a pot trial was set up using a sample of Dagun soil with a reaction value of pH 4·2. Lime was added to raise the pH to 5·0 (2 tons/acre) for one treatment, and to 6·0 (4 tons/acre) for the second treatment. It was expected that the pots treated with the lower level of lime would grow plants partly affected by the disorder and with a medium uptake of manganese. Four replications were used, making 12 pots in all. Each pot contained 25 lb. of soil.

Fig. 3.

Effect of pH on Plant Growth. Pot 3, pH 4.2; Pot 9, pH 5.1; Pot 8, pH 5.9; Pot 1, pH 4.2.

Precipitated chalk was used as the source of lime. It was mixed with the soil, and water added to keep the pots at a little under field capacity. When the soil pH had reached equilibrium (about 10 days) French dwarf bean seed (variety Brown Beauty) was planted. Fertilizer was added at the same rate as that used by most bean farmers in the Dagun district—8 cwt. of 5:14:2 mixture per acre applied in the rows. Three plants were grown in each pot.

Good growing conditions were experienced, and the plants in the no-treatment plots were showing typical symptoms within 14 days of germination. At this stage, all the plants in the other pots were healthy. Flowering commenced 5½ weeks after germination, but the plants in the treated pots were the only ones to set fruit. At this stage, the control plants were very severely affected. They had small leaves, with the usual characteristics, and were only 4–5 in. high. The plants growing in soil at pH 5·1 were no different in appearance from those at pH 5·9. The photographs illustrating the affected and healthy plants (Fig. 3) were taken when the plants were five weeks old.

Eight weeks after germination all plants were cut off at ground level, and any soil adhering to them brushed off. They were dried in a vacuum oven at 60°C. The soils were sampled at the same time and extracts prepared for analysis within a few days. The analyses carried out are given in Tables 5 and 6. Figures for plant analysis are given on a dry-matter basis. The dry weights of the plants in the treated pots are significantly higher than those in the untreated pots at the 1% level.

Table 5.

Soil Analyses in Pot Trial.

Pot No.	Treatment.	pH.	Exchangeable Manganese.	Easily Reducible Manganese.	Exchangeable Iron.
			ppm	ppm	ppm
1	Control	4·2	168	2030	13
2	Control	4·2	154	2270	12
3	Control	4·2	152	2330	9
4	Control	4·2	152	2370	7
5	2 tons $CaCO_3$/acre	5·1	6	2340	18
6	2 tons $CaCO_3$/acre	5·1	6	2060	13
7	2 tons $CaCO_3$/acre	5·2	16	2040	13
8	2 tons $CaCO_3$/acre	5·0	13	2340	18
9	4 tons $CaCO_3$/acre	5·8	10	2340	14
10	4 tons $CaCO_3$/acre	5·9	8	2380	15
11	4 tons $CaCO_3$/acre	5·9	5	2000	17
12	4 tons $CaCO_3$/acre	5·9	8	2000	18

Table 6.

PLANT ANALYSES IN POT TRIAL.

Pot No.	Plant No.	Treatment.	Dry Weight.	Total Mn.	Total Fe.	Fe/Mn.
			gm.	ppm	ppm	
1	1	Control	1·4	4300	1160	0·27
	2	Control	0·8	3800	800	0·21
	3	Control	1·6	6700	1630	0·24
2	1	Control	1·6	6200	1330	0·21
	2	Control	1·6	5800	820	0·14
	3	Control	1·2	4000	1650	0·41
3	1	Control	2·0	5400	850	0·15
	2	Control	1·1	5800	1000	0·17
	3	Control	1·4	5000	1100	0·22
4	1	Control	1·2	5100	2250	0·44
	2	Control	1·7	5400	1850	0·39
	3	Control	1·2	5700	1820	0·32
5	1	2 tons $CaCO_3$/acre	5·1	250	1670	6·6
	2	2 tons $CaCO_3$/acre	4·0	270	1220	4·5
	3	2 tons $CaCO_3$/acre	2·8	300	2160	7·2
6	1	2 tons $CaCO_3$/acre	3·0	260	1670	6·4
	2	2 tons $CaCO_3$/acre	2·0	180	800	3·3
	3	2 tons $CaCO_3$/acre	2·7	200	820	4·1
7	1	2 tons $CaCO_3$/acre	2·1	200	780	3·9
	2	2 tons $CaCO_3$/acre	3·9	160	860	5·3
	3	2 tons $CaCO_3$/acre	6·2	280	740	2·6
8	1	2 tons $CaCO_3$/acre	2·4	390	1510	3·8
	2	2 tons $CaCO_3$/acre	2·4	330	1460	4·4
	3	2 tons $CaCO_3$/acre	3·2	310	910	2·2
9	1	4 tons $CaCO_3$/acre	2·4	110	940	8·5
	2	4 tons $CaCO_3$/acre	3·2	330	1440	4·3
	3	4 tons $CaCO_3$/acre	1·2	340	2100	6·2
10	1	4 tons $CaCO_3$/acre	3·8	130	1780	13·6
	2	4 tons $CaCO_3$/acre	3·1	260	810	3·1
	3	4 tons $CaCO_3$/acre	2·4	180	890	4·9

Table 6—continued.

PLANT ANALYSIS IN POT TRIAL—*continued.*

Pot No.	Plant No.	Treatment.	Dry Weight.	Total Mn.	Total Fe.	Fe/Mn.
11	1	4 tons $CaCO_3$/acre	3·4	140	2220	15·4
	2	4 tons $CaCO_3$/acre	1·6	150	730	4·8
	3	4 tons $CaCO_3$/acre	2·0	140	1630	11·6
12	1	4 tons $CaCO_3$/acre	3·6	180	1580	8·7
	2	4 tons $CaCO_3$/acre	4·5	170	780	4·5
	3	4 tons $CaCO_3$/acre	1·7	90	780	8·6

DISCUSSION.

Manganese Values.

The uptake of manganese by the affected plants was surprisingly high—much higher than had been obtained in preliminary work—while the uptake from the soil in the limed pots was quite low. The figures for exchangeable manganese in the soil correlate well with the manganese in the plant, but the easily reducible manganese, as had been found before, remained at a high level.

The plants in the pH 5·1 series of pots were quite normal, and the figures for exchangeable manganese in this series of pots are low. However, some previous samples taken in the field had yielded higher amounts of exchangeable manganese at this pH value, and beans growing at this pH were affected. It is apparent that near the critical pH, exchangeable manganese can vary depending on some factor other than acidity.

Iron Values.

Iron was determined colorimetrically using o-phenanthroline (Sandall 1944, p. 271). Exchangeable iron in the soil was determined on the same extract as was used for manganese, and the values for the treated pots are significantly higher than those for the untreated pots at the 5% level. The number of replications, however, was small, and more analyses will be required before much weight can be given to this difference. The results for total iron in the plant are very variable and do not correlate with plant growth.

The relationship between iron deficiency and manganese toxicity has been discussed by several authors, but conflicting results are reported. Most writers state that symptoms of manganese toxicity are different from those of iron deficiency, while some report that plants injured by manganese excess responded to the application of ferrous salts. Mulder and Gerretsen (1952) summarized the papers which deal with this problem.

Sideris and Young (1949) reported that pineapples grown on manganiferous soils in Hawaii became very chlorotic, presumably due to excess manganese uptake, and that healthy leaf colours could be restored by spraying with ferrous salts.

Pineapples grown on acid areas of the Dagun soil behave similarly. It is necessary for farmers to spray with ferrous sulphate as often as 10 times a year to maintain good leaf colour, which is evidence of manganese-induced iron deficiency.

The analytical results for the pot trial do not show any relationship between iron deficiency and manganese toxicity in the plant, possibly because the figures are for total amounts of these elements present, rather than for the soluble fraction. Somers and Shive (1942) considered the ratio of soluble iron to soluble manganese to be important, and claimed that for good growth the ratio should be about 2·0. The ratios of iron to manganese given in Table 4 depend too much on the absolute amounts of manganese present to be of much value.

CONCLUSIONS.

For practical purposes, the excessive uptake of manganese by beans grown on the Dagun soil can be controlled by the application of lime in sufficient quantity to raise the pH to about 5·4.

The use of soil analysis to predict when the manganese uptake is likely to be injurious depends on a consideration of soil pH, and of exchangeable and easily reducible manganese. It appears that the value for exchangeable manganese will vary somewhat even in the same soil type and at the same pH. However, if the pH is low and the easily reducible manganese high, the soil can be considered as suspect.

Plant analysis is more reliable than soil tests as a means of predicting toxicity, and it would appear that a final proof can be obtained by the growth of French bean as an indicator plant, because the symptoms are so characteristic. Values greater than 1,000 ppm (dry-matter basis) of manganese in bean plants indicate manganese toxicity.

ACKNOWLEDGMENTS.

This study was suggested by Mr. C. R. von Stieglitz (Officer-in-Charge, Plant Nutrition Section), under whose direction the author worked. Mr. F. Chippendale (Senior Soils Technologist) gave freely from his experience in pot experiments. Statistical analysis of the results of the pot trial was carried out by Mr. P. B. McGovern (Biometrician, Division of Plant Industry). The help and advice of these officers is gratefully acknowledged.

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OVINE MASTITIS, WITH SPECIAL REFERENCE TO MASTITIS CAUSED BY *PASTEURELLA MASTITIDIS*.

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SUMMARY.

Six outbreaks of mastitis of sheep are described. Two outbreaks were caused by *Pasteurella mastitidis* and one by *Staphylococcus aureus*. In the remaining three outbreaks, *Past. mastitidis* was associated with *Corynebacterium pyogenes* in one, *Staph. aureus* in another and Staph. aureus and streptococci in the third.

INTRODUCTION.

Ovine mastitis has been reported from Europe (Lesbouyries, Berthelon and Macrides 1935), Russia (Milovzorov and Tchasnovnikov 1933), Great Britain (Leyshon 1929) and America (Marsh 1932; Smith and Harnden 1943; Tunnicliff 1949).

Many authors (Haupt 1932; Miessner and Schoop 1932; Marsh 1932; Milovzorov and Tchasnovnikov 1933; Macrides 1936; Smith and Harnden 1943; Tunnicliff 1949) have reported that *Pasteurella mastitidis* is the causative organism. Other organisms that have been incriminated are *Micrococcus ovis* (Haupt 1932), *Clostridium perfringens* (Weinberg, Forgeot and Moureu 1937) and *Corynebacterium ovis* (Rosati and Bertolino 1938). *Staphylococcus aureus* was isolated from the majority of cases of ovine mastitis in Tasmania (Dumaresq, personal communication 1948) and also from cases in New Zealand (Gilruth 1910), England (Leyshon 1929) and Italy (Boi 1940).

During the last five years material from six outbreaks has been examined at this Station. Information on these outbreaks is recorded.

HISTORY OF OUTBREAKS.

Outbreak 1.

An Inspector of Stock in south-western Queensland reported on an outbreak in 1948 as follows:—

> Seventy-two in a flock of 2,400 ewes, of mixed ages, had died and six had recovered. The incidence was highest in ewes suckling lambs 2½–4 months of age. Symptoms were swelling of the udder, stiffness of the hind legs, anorexia, fever and trembling. On autopsy the udder of one ewe was swollen, firm and showed evidence of gangrene. The supramammary lymph nodes were swollen. No other abnormality was noted.

Past. mastitidis was isolated from one of two milk samples submitted to this Station. Three millilitres of a broth culture of this organism was inoculated into the teat canal of the right half of the udder of a lactating ewe. After 48 hours the milk was thickened and showed a slight greenish tinge, but the organism was not recovered.

Outbreak 2.

This outbreak occurred in 1948 on another property in south-western Queensland. The Sheep and Wool Adviser reported as follows:—

> The lambs were marked and vaccinated against contagious ecthyma between 14th October and 6th November. Two days after the completion of these operations, some ewes would not allow the lambs to suckle, and on examination scabs were found on the teats. About 70 ewes died, and all of these animals had discoloured udders. The mortality was investigated on 24th November. Examination of one ewe, which was dull and standing in the shade, showed that the left half of the udder was black on the ventral portion and the remainder was discoloured. The medial portion of the right half was purple, and the affected parts were firm to the touch. On autopsy the internal organs were normal.

Pipettes of the liver, mesenteric lymph node and udder yielded *Staph. aureus*. Histopathological examination of the mammary gland showed an acute mastitis with hyperaemia, oedema and haemorrhage. There was massive necrosis, marked accumulations of neutrophils, and plugging of the alveoli with exudate and cellular debris.

Outbreak 3.

This outbreak occurred in 1952 and involved 6- and 7-year-old ewes yarded for shearing. They were in fair condition and had 4-month-old lambs at foot.

About 40 ewes and a number of lambs died. The Sheep and Wool Adviser reported the following observations on one ewe:—

> Symptoms were listlessness, laboured breathing and moist discharge from both nostrils. The left half of the mammary gland was bluish red, and there was a black scab on the end of the teat. The medial portion of the right half of the gland was dark pink. A swelling 8 inches long and 5 inches wide was present anterior to the udder.

Corynebacterium pyogenes was isolated from the left half of the udder and *Past. mastitidis* from the right half.

Outbreak 4.

In 1952 a 4-tooth ewe in a flock of 350 Merino ewes with 1–2-month-old lambs at foot was noticed lame. Three days later this animal was examined by a Sheep and Wool Adviser, who reported as follows:—

> The ewe had anorexia and was dull. The left side of the udder was hot, red and swollen, and the swelling extended 6 inches along the abdomen. The other side of the udder contained no milk, but was normal on palpation. This ewe responded to two subcutaneous injections at twelve-hour intervals of sodium sulphamezathine (33⅓ per cent. solution) at the rate of 1 ml. per 5 lb. body weight. Two further cases of mastitis occurred in this flock, but no treatment was given, and one ewe died.

Past. mastitidis, *Staph. aureus* and streptococci were recovered from the milk sample submitted to the laboratory.

Outbreak 5.

A ewe from the experimental flock at this Station was culled in 1953 and autopsied because of old age, faulty teeth and emaciation. One half of the udder was distorted and firm and contained irregular cavities filled with greenish-yellow semi-fluid pus. Fibrous tissue had replaced almost all of the remaining mammary tissue. *Past. mastitidis* was recovered from the pus.

Another ewe in good condition was found dead. On autopsy both halves of the udder were enlarged and firm, the cut surface showing mottling with small areas of fibrosis. Brownish fluid was present. *Past. mastitidis* and *Staph. aureus* were isolated from the diseased tissues.

Outbreak 6.

This occurred in 1953 in a flock of 100 stud ewes, 2–8 years old with 2–4-week-old lambs at foot. The first symptoms noted were lameness and swelling of one half of the udder, the skin being bluish-black. Of seven cases seen, six died. There was a history of cases having been seen in previous years on this property. When recovery occurred there was sloughing of the affected half of the udder.

Past. mastitidis was isolated from milk samples from each of three ewes. The milk was green, contained many clots and films showed numerous leucocytes, lymphocytes predominating. A 24-hour broth culture injected into the left teat canal produced an acute mastitis with later sloughing of that half.

BACTERIOLOGICAL RESULTS.

The bacteria isolated from the six outbreaks are listed in Table 1.

Table 1.

BACTERIA ISOLATED FROM SIX OUTBREAKS OF OVINE MASTITIS.

Outbreak.	Bacteria.
No. 1	*Past. mastitidis*
No. 2	*Staph. aureus*
No. 3	*Past. mastitidis, C. pyogenes*
No. 4	*Past. mastitidis, Staph. aureus,* streptococci
No. 5	*Past. mastitidis, Staph. aureus.*
No. 6	*Past. mastitidis*

The strains of *Staph. aureus* were Gram positive cocci and produced typical colonies and haemolysis on sheep's blood agar.

The *C. pyogenes* strains were Gram positive diphtheroid bacilli, which produced acid in glucose, maltose, sucrose, mannitol and lactose, liquefied Loeffler's inspissated serum and produced haemolysis on sheep's blood agar. The single strain of streptococci was not identified.

The strains of *Past. mastitidis* were all Gram negative, non-motile, non-sporing bacilli. After 24 hours' incubation at 37°C., colonies on 10% sheep's blood agar were 2–3 mm. in diameter, greyish, round, convex and entire. A zone of haemolysis usually occurred beneath the colony. The biochemical characteristics are given in Table 2. As cultures were not retained, only those characteristics tested at the time of isolation are given. Acid production was often slow, particularly with lactose medium. Andrade's indicator was used in carbohydrate media, and incubation was for at least seven days at 37°C.

The strain from outbreak 6 was non-pathogenic for mice, guinea pigs and rabbits when inoculated intraperitoneally. One rabbit inoculated with 1 ml. culture intravenously was not affected.

DISCUSSION.

Miessner and Schoop (1932) differentiated two clinical forms of this disease—*mastitis infectiosa* caused by *Past. mastitidis* and *mastitis gangraenosa* produced by *Past. mastitidis* plus staphylococci or by staphylococci alone. However, other species of bacteria rapidly become established in the damaged mammary tissue, particularly in mastitis caused by *Past. mastitidis* (Lesbouyries, Berthelon and Macrides 1935; Tunnicliff 1949). *C. pyogenes* in particular is often a secondary invader and causes severe damage of the udder tissue. Hence, specimens for bacteriological examination should be taken from early cases.

The economic loss caused by ovine mastitis, particularly in Australia, is difficult to assess, but the absence of reports suggests that it is relatively rare. Tunnicliff (1949), in an excellent review of pasteurella mastitis in ewes in the United States, stated that his records showed that over a period of 17 years, 2–3% of 19,550 lambing ewes developed mastitis. The mortality in these ewes was 21%.

Carroll (1949) and Belschner (1950) described mastitis in Australian sheep but gave few details of the etiology of the disease.

That ovine mastitis may be a cause of economic loss is suggested by data collected by Moule (1954) during a survey of neo-natal mortality of lambs. He stated that 351 out of 1,587 ewes had abnormal udders. Although some of these were attributed to chronic infection, no information on the number of such cases or etiology was given. Miessner and Schoop (1932) considered that surviving ewes may not be suitable for breeding purposes because they may be unable to support a lamb. In outbreaks of pasteurella mastitis, pneumonia caused by the same organism often occurs in the lambs (Haupt 1932; Miessner and Schoop 1932; Lesbouyries, Berthelon and Macrides 1935).

There was no apparent common predisposing factor in the outbreaks described by us. Marsh (1932) suggested that bruising of the udder by large lambs may be a predisposing factor, while Miessner and Schoop (1932) noted that hungry lambs were often the cause of wounds through which infection could enter. The latter authors stated that infection occurred irrespective of the standard of hygiene maintained. In our outbreak 2 there was a possibility that the ewes had teat lesions caused by contagious ecthyma following vaccination of the lambs. These lesions would be favourable sites for the entry of the staphylococci. Dumaresq (1948) recorded sores and scabs on the tips of the teats in up to 60% of maiden Merino ewes, but the etiology of these lesions was not known.

Opinions differ on the value of immunisation for the prevention of pasteurella mastitis. Carefully killed cultures were used by Miessner and Schoop (1932) to vaccinate 800 ewes. Results indicated that a considerable degree of immunity was produced, particularly in ewes given three injections of vaccine. Also the disease was milder in vaccinated than in non-vaccinated animals. Lesbouyries, Berthelon and Macrides (1935) used Miessner and Schoop's method of vaccination but no definite conclusions could be drawn from their results. Tunnicliff (1949) found that formolised suspensions were not satisfactory as immunising agents, but that a living avirulent strain produced approximately 50% immunity against severe artificial infection. The viability of the living vaccine was not long enough to allow effective field application.

Formolised autogenous vaccines produced a favourable response in the treatment of pasteurella mastitis in sheep and goats but did not prevent the gland becoming functionless (Smith and Harnden 1943).

Table 2.

Biochemical Characteristics of Five Strains of *Past. mastitidis*.

	Strain.				
	1.	3.	4.	5.	6.
Arabinose ..	—	A		A (L)	A (L)
Xylose	A	A	A (S)	A	A
Rhamnose ..	—	—	—	—	—
Glucose	A	A	A	A	A
Fructose ..	A	A	—	A	A
Mannose ..	—	A	—	—	—
Galactose ..	A (S)	A	A	A	A
Sucrose	A	A	A	A	A
Maltose	A	A	A	A	A
Lactose	A	A	A (L)	A (L)	—
Trehalose ..		A		—	
Raffinose ..	A	A		A	A
Starch	—	—		—	A
Inulin	—	—		—	—
Dextrin		—		—	—
Glycogen ..		—		A (S)	—
Glycerol ..	A	A	—	A	A
Adonitol ..	—	—		—	—
Mannitol ..	A	A	A	A	A
Dulcitol	A (S)	—	—	—	—
Sorbitol	A	A	A	A	A
Salicin	A (S)	—	—	—	—
Inositol	—	A		A	A
Indole	—	—	—	—	—
H_2S		—		—	—

Key. — = negative; A = acid production; A (L) = late acid production; A (S) = slight acid production

As sheep are not usually watched closely for sickness under Australian conditions, the rapidly developing disease is often too advanced for treatment to be beneficial. Antiserum prepared in horses was not satisfactory in the treatment of ewes (Miessner and Schoop 1932). Acriflavine given intravenously did not cure one experimental or 13 natural cases (Marsh 1932). Sulphamezathine given orally and intravenously produced favourable response in experimental cases, but treatment was ineffective unless started within 24 hours of the appearance of symptoms (Tunnicliff 1949).

Measures for the control of mastitis due to *Past. mastitidis* should include the slaughter of survivors. Tunnicliff (1949) described a case where an experimentally infected ewe carried the organism for 10 months without any clinical evidence of infection until handling of the udder produced acute mastitis. Avoiding hunger and thirst of the lambs may prevent udder damage and so decrease the chance of infection (Miessner and Schoop 1932).

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FOLLICLE GROUPS IN THE SKIN OF A MOSAIC MERINO SHEEP.

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SUMMARY.

The section of skin growing lustre type wool had a ratio of secondary to primary follicles approximately half that of the skin growing normal wool. The section had fewer primarily follicles and the size of the follicles was markedly larger.

INTRODUCTION.

Although somewhere in the vicinity of one hundred million sheep are shorn in Australia each year, comparatively few fleeces showing gross abnormalities seem to be brought to the notice of laboratories working on fleece improvement.

Sheep and wool classers recognise that there may be a transition from one wool quality to another in various parts of the fleece. These changes are usually gradual, although extremes may occur on the breech, or upon the neck folds of Merino rams.

Our attention was drawn recently to a Merino sheep showing mosaicism. As far as is known, the factors responsible for the development of this condition have not been determined. However, previous observers do not seem to have studied the distribution of follicle groups in the skin of mosaic animals. This paper reviews the work of previous observers and records the results obtained from examination of material which has recently become available.

REVIEW OF LITERATURE.

Ross (1933) described mosaicism in an animal from a flock in which Merino rams had been used for the previous 30 years. The fleece he saw was segregated into two distinct wool types, one being classified on character as a Lincoln wool of 46's count and the other as a finer wool of 64-66's count. The coarser wool had a staple length of $5\frac{1}{2}$ in. and mean fibre diameter of 31·2μ. The fine wool had a staple length of 4 in. and a mean fibre diameter of 21·7μ.

Kelley (1939) described two cases of mosaicism. One ewe had one area of coarse fibres confined to the loins. On the other there were extensive, though irregular, areas on each side of the sheep which grew fibres with fewer crimps per inch than the remainder of the fleece. The greater part of the fleece of the former animal was classed as a 74's spinning count and the mean fibre diameter was 19·6 μ. The coarser fibres were classed as a 58's spinning count and their mean fibre diameter was 26·8μ.

Although the wool of the latter ewe was considered to vary between 56's and 70's spinning counts, it was found that there were only small differences in the diameters of fibres from different parts of the fleece. Histological examination of skin specimens from areas growing abnormal and normal wool revealed that the skin growing the coarser wool contained approximately half the number of follicles to be seen in the skin growing normal wool.

Bosman (1935) described a Merino sheep which had a distinctive band, about 3 in. wide, of long coarse wool growing transversely across the back and stretching over the sides towards the belly. Fibres from the abnormal part of the fleece measured 20·2 μ in diameter, while those from normal parts of the fleece measured 17·7 μ in diameter.

Lang (1952) reported examining the fibre diameters and fibre lengths of two distinct wool types growing on adjacent areas of the same sheep. One was a lustrous type of wool which had a mean fibre diameter of 27·0 μ and the other Merino type with a thickness of 18·2 μ.

Carter (1939, 1942) described a method for studying the distribution of follicle groups in the skin of Merino sheep and its application. The development and general histology of the follicle group in the skin of the Merino was described by the same author (Carter 1943), and the hair follicle group and its topographical variations in the skin of the Merino foetus were described by Carter and Hardy (1947).

Carter (1951) recorded variations in the ratio of secondary to primary follicles in different breeds of sheep. It commonly varied between 14:1 and 22:1 for Merino sheep and fell to as low as 5:1 or 4:1 for long-woolled sheep of the British breeds.

MATERIAL.

A sample of abnormal wool taken from the wither of a wether in Central Queensland was submitted to the Wool Laboratory by the representative of a woolbroking firm. Enquiries established that it came from the same sheep which grew the abnormal wool described by Lang (1952). The difference in the wool from the abnormal area and from an adjacent part of the fleece can be readily seen (Fig. 1).

Samples of skin were obtained from both the areas of abnormal and adjacent normal wool growth. These were sectioned histologically, and examined microscopically to determine the ratio of secondary to primary follicles.

The histological technique employed in the preparation of the skin samples was similar to that used by Carter (1939) with a few slight modifications.

Fig. 1.

Lustre Type and Normal Merino Wools that Grew on Adjacent Areas on the Wither of a Merino Wether.

After being fixed in 5% formol saline for at least six days, any muscle and excess wool were trimmed off with scissors. The skin then went through the stages of dehydration and clearing into wax baths on an automatic tissue processor. A xylol-alcohol mixture was used as a sub-clearing agent, while the final clearing was done in two changes of xylol. The sample was impregnated with paraffin for three hours and finally in a mixture of 3% beeswax in paraffin wax for six hours before embedding. No attempt was made to prevent the skin from shrinking during processing, as the subsequent examination was primarily for ratio of secondary to primary follicles, and not for follicle population density.

During embedding, the skin was held firmly but not tightly between a glass slab and a small backing piece of glass by means of a clamp. This remained in position until the beeswax/paraffin wax mixture set. A warmed, rather than cooled, glass slab was found to be an advantage when the clamp was used.

Sections of 8μ in thickness were cut in series and mounted using an egg albumen/glycerin adhesive. These were stained with a modification of Mayer's acid haematoxylin, counterstained with an eosin-phloxine mixture and selectively stained with picric acid.

The examination of the sections was performed at a magnification of 115 x. The images of fibres falling within a square of 11·5 cm. were outlined for 18 fields on each sample. Follicles were distinguished as primary or secondary by the accessory structures described by Carter (1939). The number of each type of follicle was counted and the ratio of secondary to primary follicles calculated for each sample.

Samples of normal and abnormal wool were examined for medullated fibres by viewing them in transmitted light while immersed in orthodichlorbenzene.

RESULTS.

The results of fibre diameter measurement and skin sample examination are listed in Table 1.

Table 1.

Sample.	Mean Fibre Diameter (microns).	Standard Deviation (microns).	Coefficient of Variation (%).	Range (microns).	Rates of Secondary to Primary Follicles (Mean of 18 fields for each Sample).	Percentage of Primary Follicles.
SHEEP 1:						
Normal area ..	18·2*	3·1†	17·1*	24†	17·3	5·5
Abnormal area ..	27·0*	6·5†	24·0*	28†	10·3	8·7

* Lang (1952) † Calculated from results*.

No medullated fibres were detected in either the normal Merino type or the lustre type wools comprising the specimen submitted by the representatives of the woolbroking firm.

DISCUSSION.

The ratio of secondary to primary follicles in the circumscribed area of skin growing the lustre type wool was approximately half that of the adjacent area on which normal wool grew. There were also fewer primary follicles in the skin carrying the abnormal wool than in that growing wool with normal Merino characteristics. The size of the follicles was also markedly larger.

Plates 2 and 3 are microphotographs at a magnification of 96x of sections cut from the skin growing abnormal and normal wools. These show the difference in the size and numbers of follicles and in the arrangement of follicle groups in the two areas.

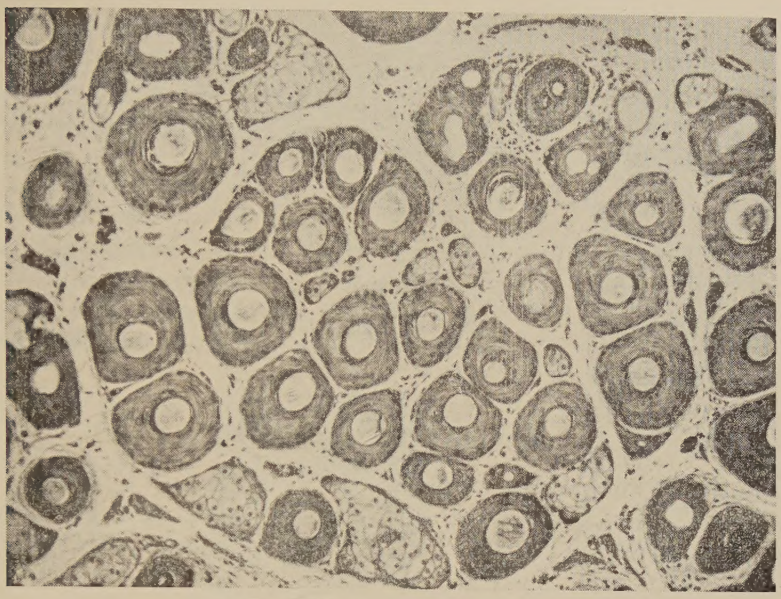

Fig. 2.

Microphotograph of a Section Cut from the Skin Growing Abnormal Wool. The fewer follicles, both primary and secondary, and the larger size of the fibres and follicles in the abnormal area can be seen by comparing Figs. 2 and 3. The average S/P ratio of 18 fields of the abnormal area is 10.3:1. (x96).

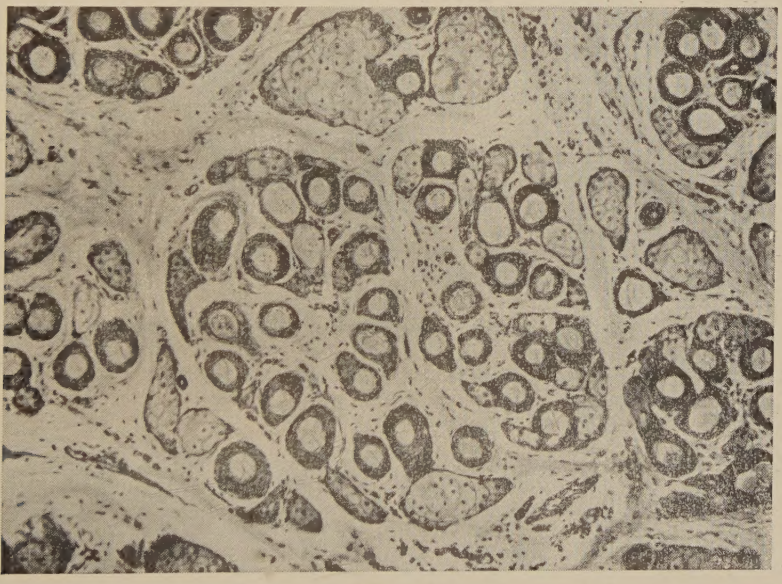

Fig. 3.

Microphotograph of a Section Cut from the Skin Growing Normal Merino Wool Adjacent to the Area of Abnormal Wool Growth. By comparison with Fig. 2, the smaller sizes of the fibres and follicles and their larger number are quite apparent. The average S/P ratio of 18 fields of this normal area is 17.3:1. (x96.)

Kelley (1939) suggested that mosaicism in sheep results from an interference during the later stages of embryonic development in the influence of certain chromosomes on cells associated with the growth of wool fibres. If this hypothesis is correct, the results reported in this paper indicate that the distribution, size and arrangement of the follicle groups are affected. The ratio of secondary to primary follicles in the skin growing the lustre type wool approximates closely to that which Carter (1951) considers to be normal to long-woolled sheep of the British breeds. In general appearance the lustre type wool is similar to that seen in British long-woolled breeds, except that it is somewhat shorter in length. The average fibre thickness of 27·0 μ is, however, finer than is normal for the British long-woolled breeds.

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